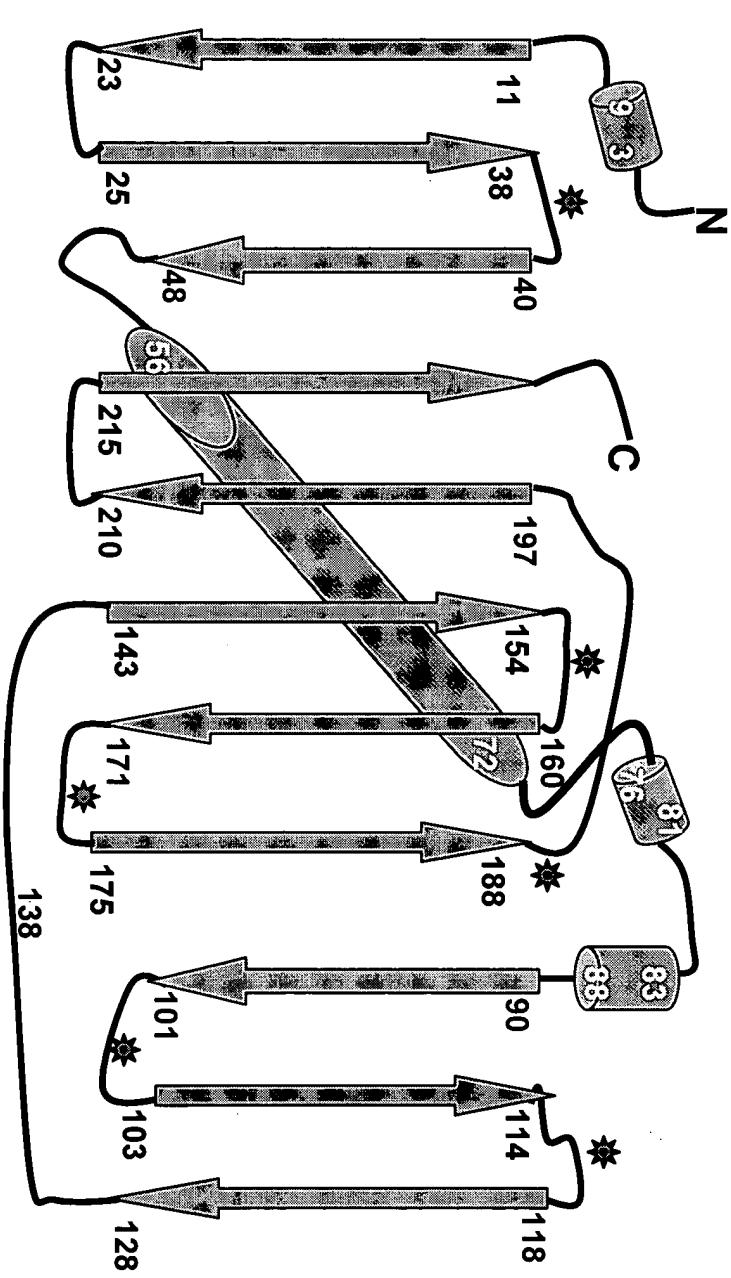


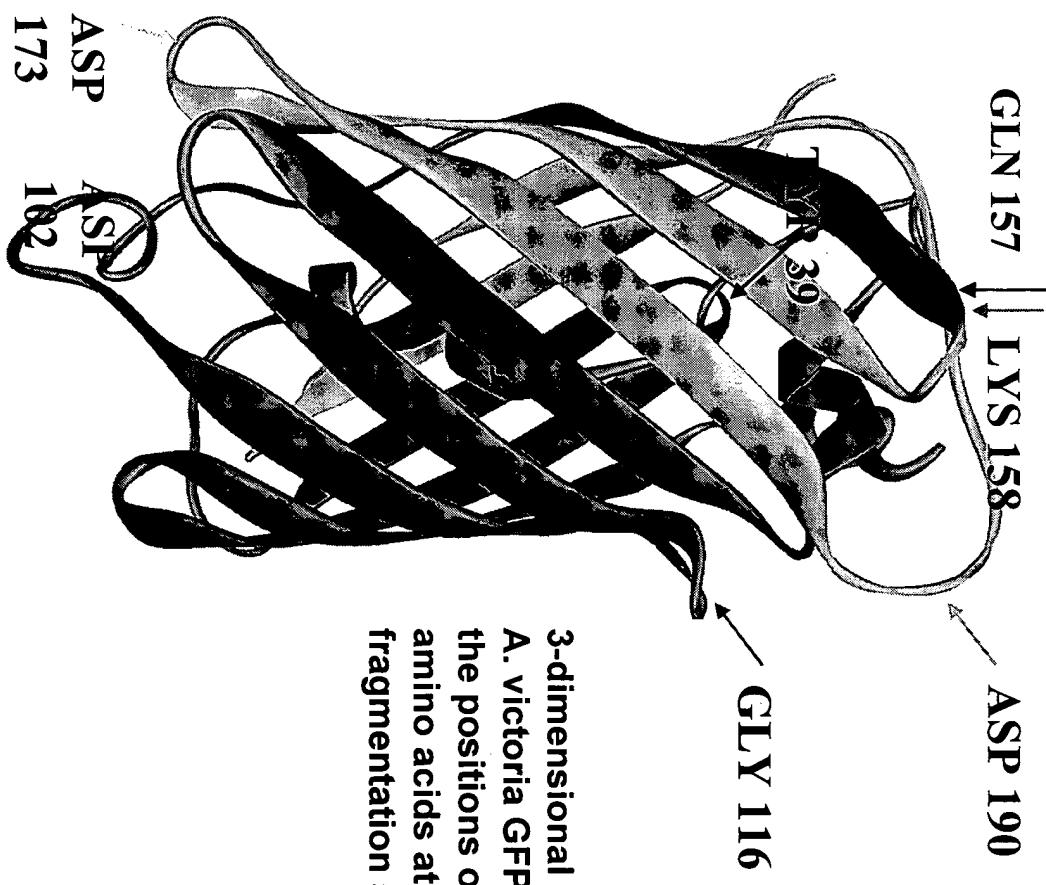
Fig. 1a



Aequorea Victoria Green Fluorescent Protein (GFP)

\* Loops highlighted by asterisks designate alternative fragmentation sites that are the subject of the invention, comprising amino acid residues 38-40 (region 1); residues 101-103 (region 2); residues 114-118 (region 3); residues 154-160 (region 4); residues 171-175 (region 5); and residues 188-190 (region 6).

**Fig. 1b**



3-dimensional structure of  
A. victoria GFP showing  
the positions of specific  
amino acids at 6 alternative  
fragmentation sites

**Fig. 2**

1. Select a fluorescent protein and its corresponding DNA sequence
2. Create fragments and mutant fragments by:
  - A. Rational design, followed by genetic engineering or oligonucleotide synthesis; and/or
  - B. Random or directed fragmentation of full-length DNA molecule
3. Create 5' or 3' fusions of fragments (F1,F2) with genes encoding other proteins (A,B) in expression vectors
4. Co-transfect fragment pair fusions into cells; allow expression of fusion proteins A,B
5. Measure fluorescent signal, e.g. by fluorometry, FACS, or microscopy

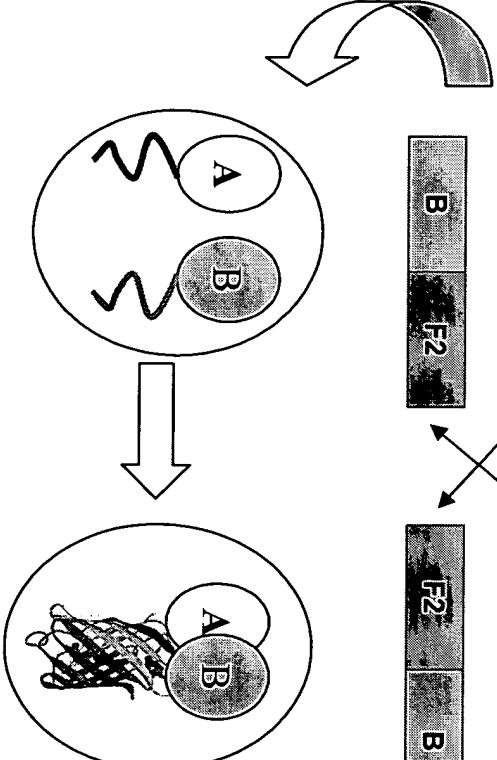
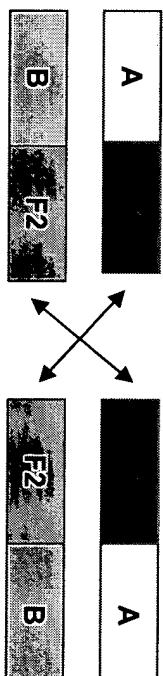
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 TAAACTATAACTCACACATGTATACATCATGGCAGACAAACAAAGATGGATCAAG  
 TAACTCTAAATAGACACACATGTAGAGATGGGAAGGATGGAAACATTCTGGACACA  
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 TGAACAGCTGCTGGATTACACATGGCATGGATGAACTATACAA

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 TGATTTAAGAAGATGGAACATCTGGACACAAATTGGATAACTATACT  
 CACACATGTATACATCATGGCAGACAAACAAAGATGGATCAAGTAACT  
 CAAATAGACACACATGTAGAGATGGGAAGGCGTTCACTAGCAGACACATTCAA  
 CAAATACTCCAATGGGATGGCCCTGCTCTTACACAGACACACATTACCTGT  
 CCACACAACTCTGCCCTTCAAGATGCCACAGAAAGAGAGACACATGGCC  
 TCTGAGTTAACACAGCTGCTGGATTACACATGGCATGGATGAACTATACAA

F2

F1

F2

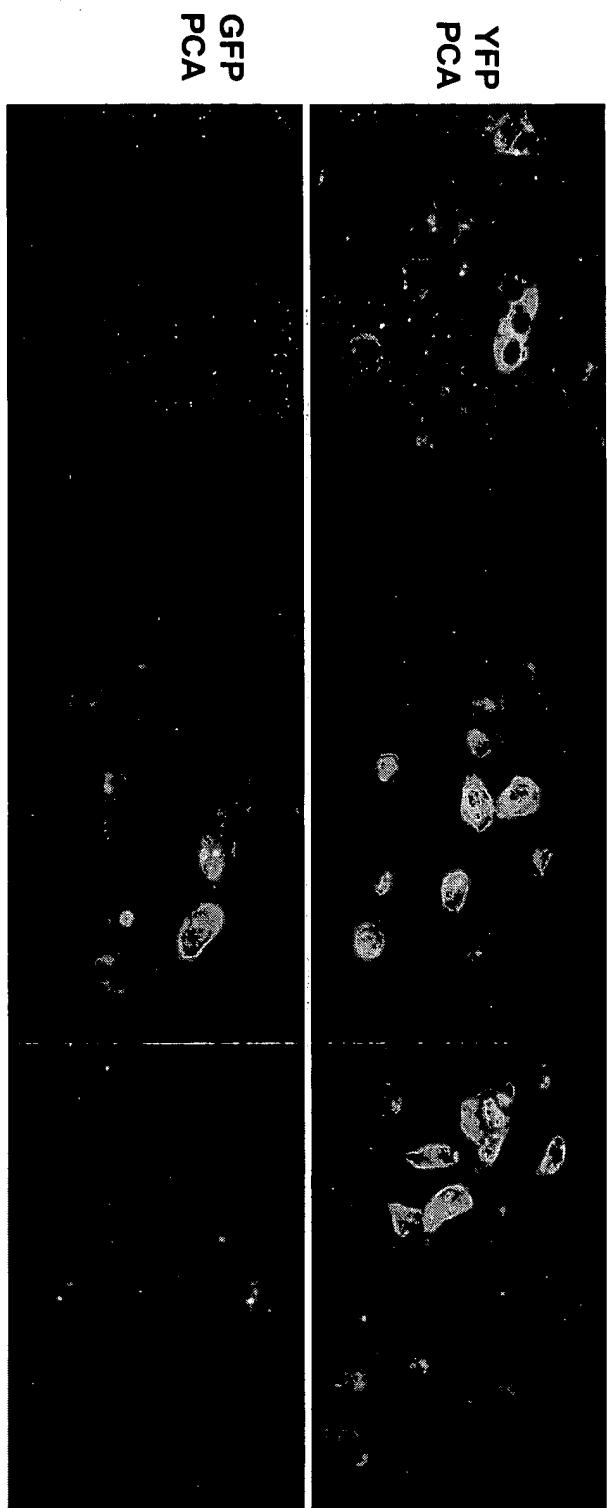


**Fig. 3**

**p53/p53**

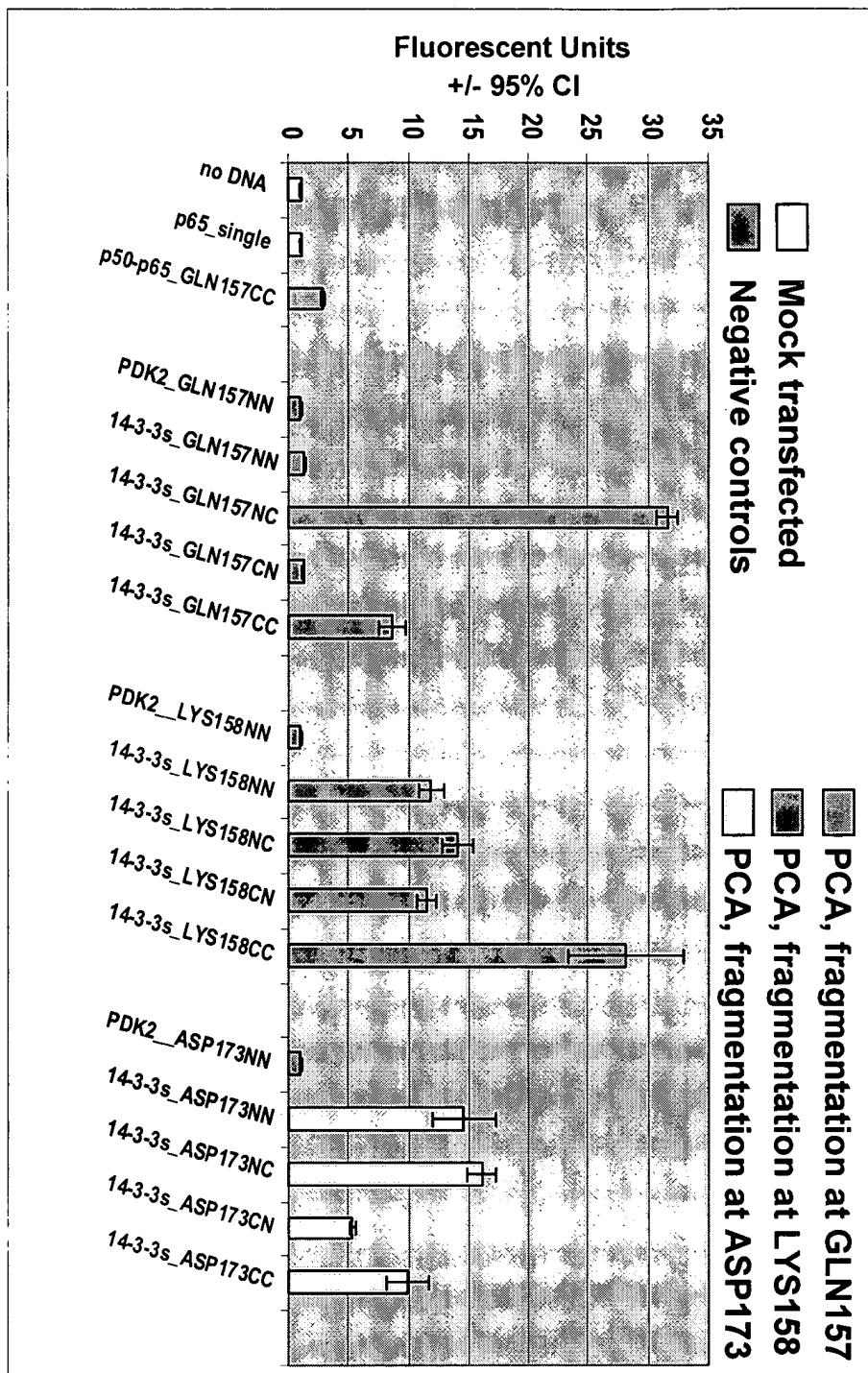
**E6/E6AP**

**E6/p53**

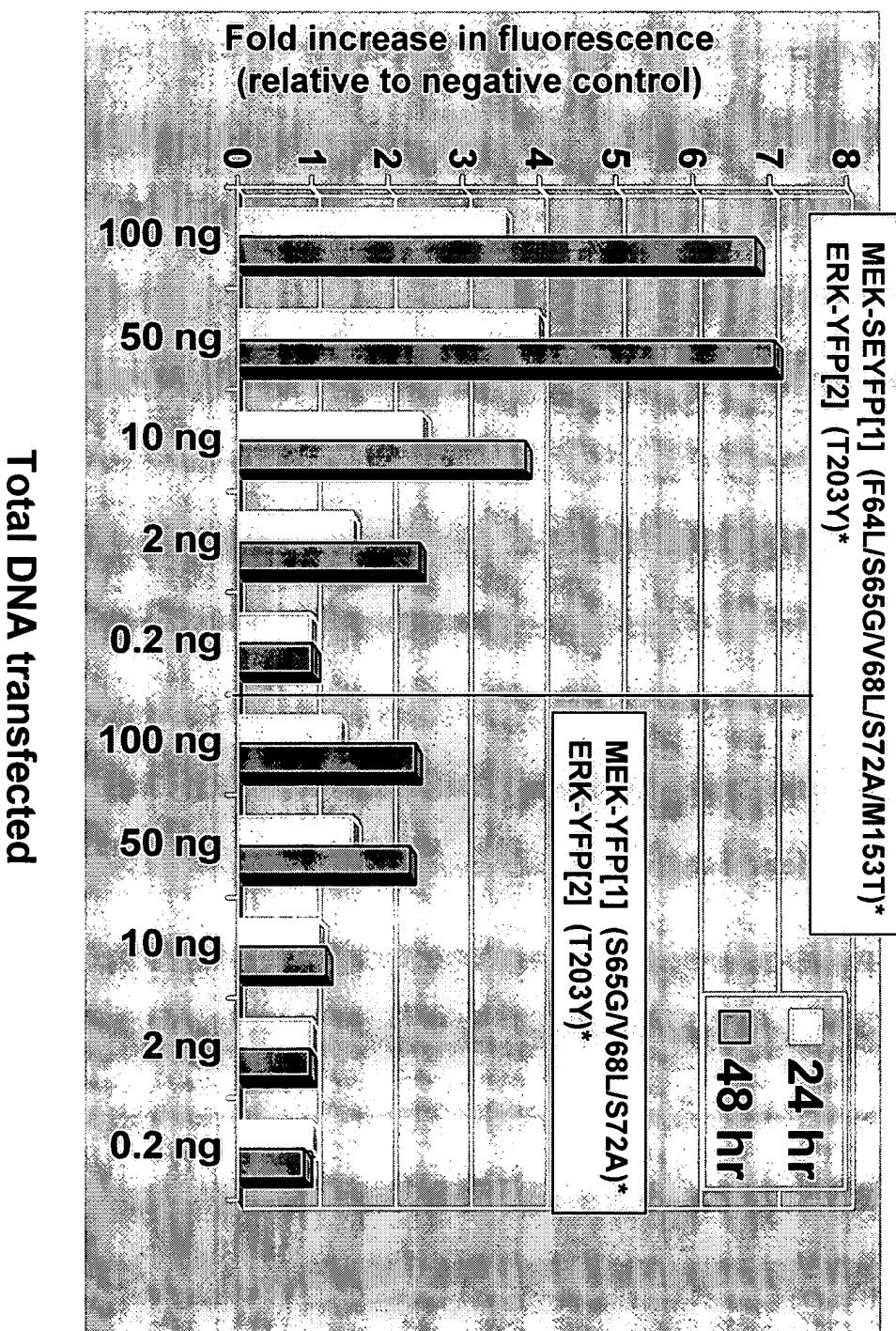


**24 Hour Expression in HEK293E Cells (1.2 sec exposure)**

**Fig. 4**  
**Examples of PCAs based on alternative fragmentation sites of YFP**



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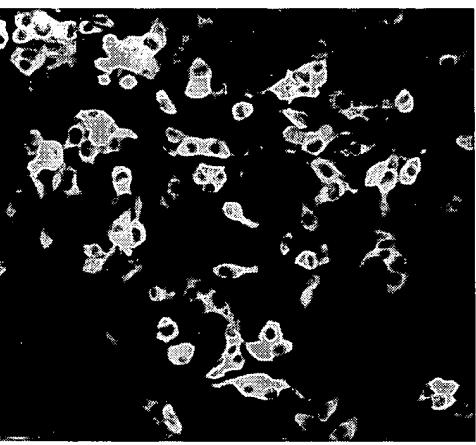


\* Note: amino acid changes are designated relative to wild-type GFP

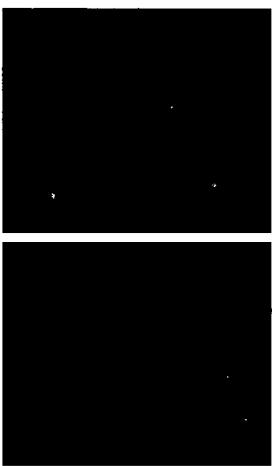
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Fig. 6

a. MEK-YFP[1] (F64L/S65G/V68L/S72A/M153T)\*  
ERK-YFP[2]-T203Y\*



C. Single fragment control:  
Pdk2-YFP[1] (F64L/S65G/V68L/S72A)



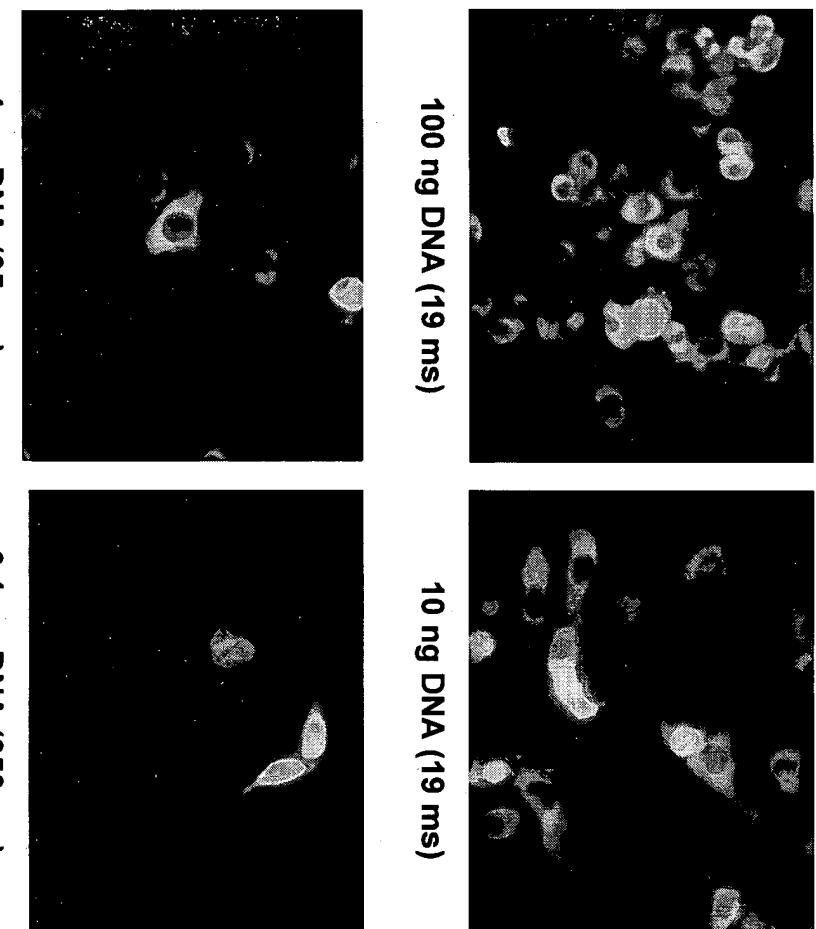
b. MEK-YFP[1] (S65G/V68L/S72A\*)  
ERK-YFP[2] (T203Y\*)

d. Single fragment control:  
Pdk2-YFP[2] (T203Y)



\* Note: amino acid changes are  
designated relative to wild-type GFP

**Fig. 7a**  
An intense fluorescent PCA (IFP PCA) based on mutant fragments  
of YFP



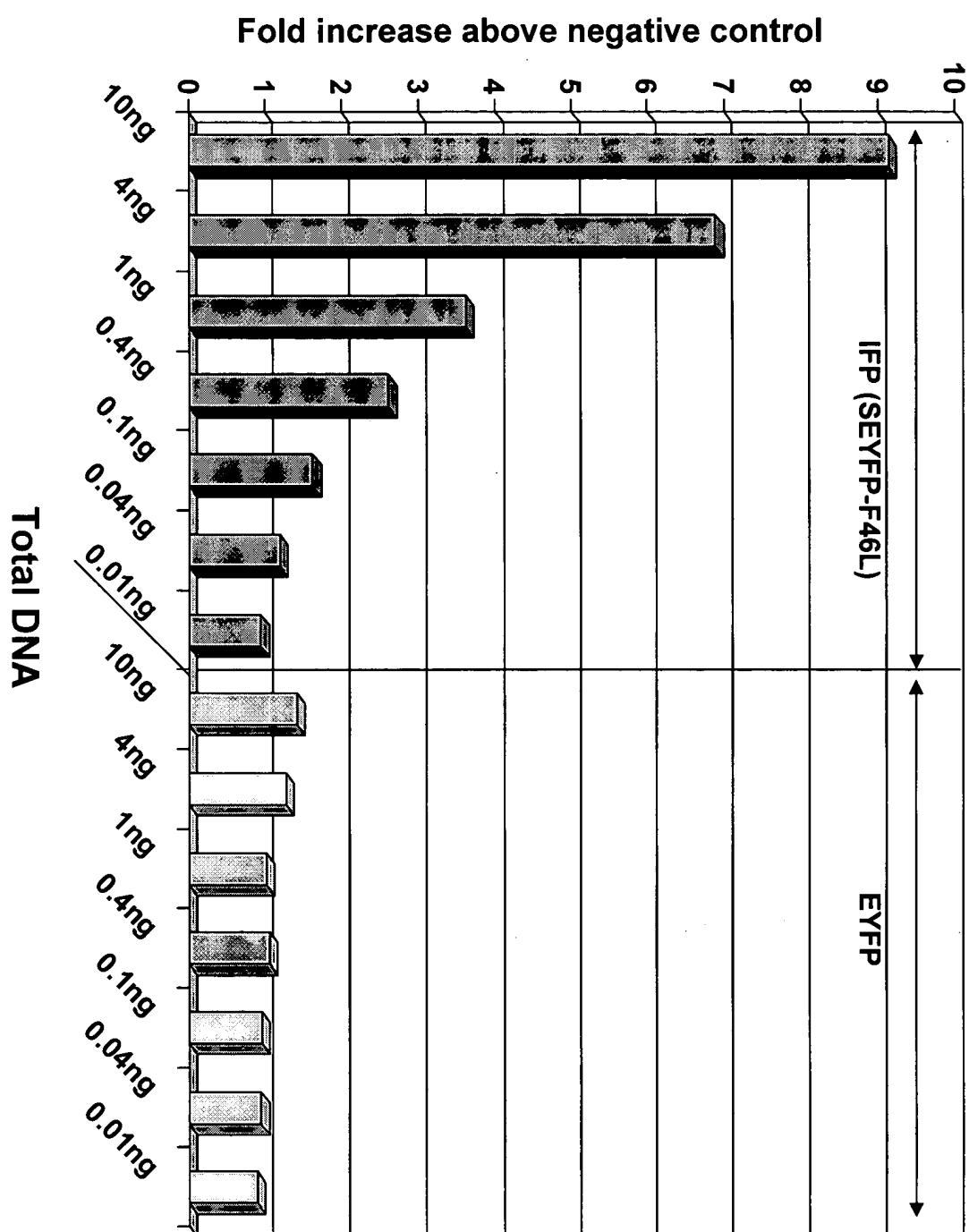
**Results shown are for MEK-IFP[1] + IFP[2]-ERK**

IFP[1]: F46L/F64L/S65G/V68L/S72A/M153T

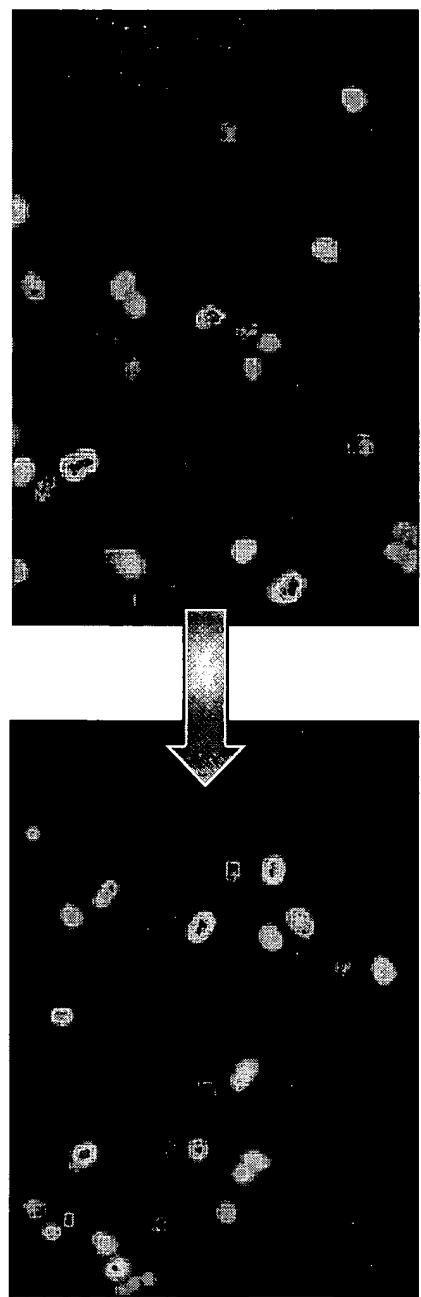
IFP[2]: V163A/S175G/T203Y

*Note: amino acid changes are designated relative to wild-type GFP*

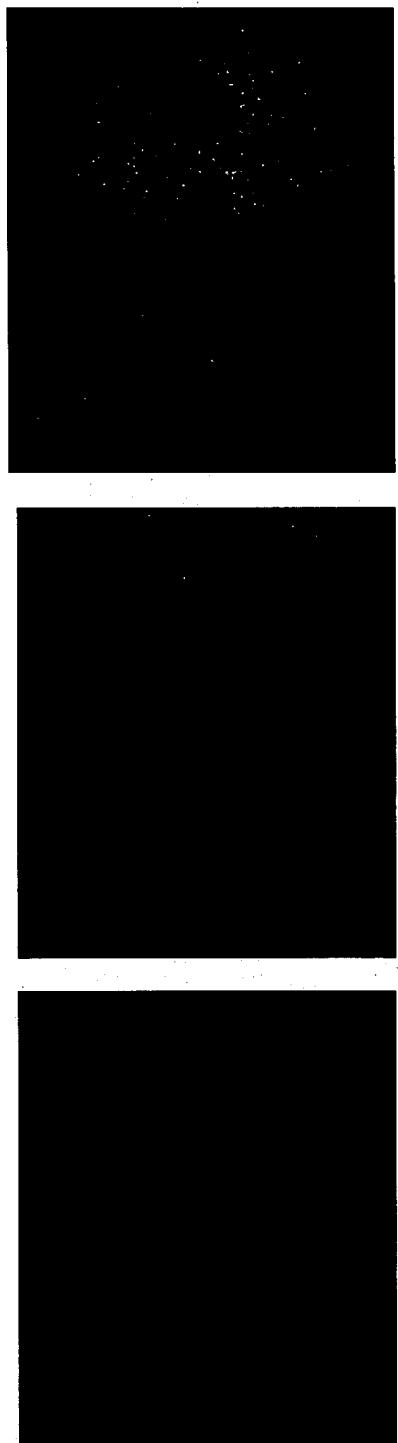
**Fig. 7b.**  
Enhanced PCA signals based on mutant fragments:  
IFP (SEYFP-F46L) versus EYFP



**Fig. 8**  
High-content assays based on IFP PCA:  
cytokine-dependent translocation of p65/p50



**Fig. 9 .**  
**Spectrally shifted (blue) PCAs based on mutant fluorescent protein fragments**  
**(excitation= 436 nm, emission= 480 nm )**



14-3-3 $\sigma$ /14-3-3 $\sigma$

p65/p50

Negative Control

**Fig. 10.**  
Multi-color protein-fragment complementation assays (PCA)

